Fisheries and Oceans Canada | Pêches et Océans Canada
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Canadian Science Advisory Secretariat (CSAS)

Research Document 2014/069

Maritimes Region

Within- and among-population genetic variation in Outer Bay of Fundy Atlantic Salmon (*Salmo salar* L.), with special emphasis on the Saint John River system in the context of recent human impacts

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November 2014

Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Research documents are produced in the official language in which they are provided to the Secretariat.

Published by:

Fisheries and Oceans Canada
Canadian Science Advisory Secretariat
200 Kent Street
Ottawa ON K1A 0E6

http://www.dfo-mpo.gc.ca/csas-sccs/
csas-sccs@dfo-mpo.gc.ca

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ISSN 1919-5044

Correct citation for this publication:

O'Reilly, P.T., Jones, R., and Rafferty, S. 2014. Within- and among-population genetic variation in Outer Bay of Fundy Atlantic Salmon (*Salmo salar* L.), with special emphasis on the Saint John River system in the context of recent human impacts. DFO Can. Sci. Advis. Sec. Res. Doc. 2014/069. vi + 34 p.

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ABSTRACT

Recent declines in the number of adult Atlantic Salmon (*Salmo salar*) returns to rivers of the Outer Bay of Fundy (OBoF), and low probability of population rescue by conspecifics from neighbouring Designatable Units (DUs), have precipitated the recent designation of OBoF Atlantic Salmon as endangered by the Committee on the Status of Endangered Wildlife in Canada. Although a number of factors are likely involved in historic and the more recent reductions in anadromous runs in the OBoF, there has been considerable focus on the direct role of hydroelectric facilities on the Saint John River (SJR) system, and possible associated effects of large-scale mitigation stocking that commenced in the early 1970s, including loss of within-population genetic variation and the homogenization of putative tributary stocks above Mactaquac Dam and the resulting loss of local adaptation.

This research document will report on results of analyses of two molecular genetic datasets (originally collected for other purposes), one involving eight small sample collections obtained from the OBoF analyzed at a limited set of seven microsatellite loci, and another involving only two OBoF locations, but analyzed at a larger set of 17 microsatellite loci. Both datasets include at least one tributary of the SJR above and one below Mactaquac Dam, and multiple reference populations from other DUs.

Overall, little evidence of marked reductions in genetic diversity in tributaries above Mactaquac Dam (potentially impacted by reductions in census and effective population sizes) relative to those below Mactaquac Dam was found. Indeed, levels of variation within OBoF sample collections overall are comparable to those obtained from large populations in the Gaspé-Southern Gulf of St. Lawrence DU and elsewhere, and considerably greater than many sample collections obtained from the Inner Bay of Fundy and the Southern Upland DUs.

Additionally, statistically significant differences in allele frequency distributions were observed between two sets of sample collections obtained from locations above Mactaquac Dam, suggesting that potentially heightened stocking-mediated gene flow may not have completely homogenized populations above Mactaquac Dam. On the other hand, levels of genetic structuring across tributaries above Mactaquac Dam were lower (approximately half) compared to sample collections obtained from tributaries below Mactaquac Dam. Although these results may reflect the effects of stocking-related increases in gene flow among upper SJR tributaries, similar patterns (greater differentiation among lower river tributaries compared to upper river tributaries) have also been observed in other large (and less impacted) river systems, indicating that it is also possible that patterns in the SJR system may reflect natural biological processes.

Results presented here, and similar findings on the impacts of stocking on homogenization and loss of local adaptation in other endangered populations of Atlantic Salmon (discussed in this document), indicate that although the potential risks of hatchery stocking on wild populations are substantial, findings of even large-scale, long-term stocking, involving local and/or non-local salmon, cannot alone be taken as evidence that complete homogenization of wild populations has taken place, that extensive loss of local adaptation and fitness has occurred, and that conservation measures aimed at protecting remaining populations are not warranted.

Diversité génétique au sein des populations et entre les populations de saumons de l'Atlantique (*Salmo salar* L.) de l'extérieur de la baie de Fundy, en particulier dans le réseau hydrographique du fleuve Saint-Jean dans le contexte de répercussions récentes de l'activité humaine

RÉSUMÉ

Les diminutions récentes du nombre de montaisons de saumons de l'Atlantique (*Salmo salar*) adultes dans les rivières de l'extérieur de la baie de Fundy et une faible probabilité de sauvetage de cette population par des congénères des unités désignables (UD) voisines ont accéléré la désignation récente du saumon de l'Atlantique de l'extérieur de la baie de Fundy comme espèce en voie de disparition par le Comité sur la situation des espèces en péril au Canada (COSEPAC). Bien qu'un certain nombre de facteurs puissent expliquer les diminutions passées et plus récentes du nombre de montaisons de poissons anadromes dans les rivières de l'extérieur de la baie de Fundy, l'accent a surtout été mis sur le rôle direct des installations hydroélectriques en place sur le réseau hydrographique du fleuve Saint-Jean, de même que sur les effets combinés de l'atténuation à grande échelle de l'empoissonnement qui a commencé au début des années 1970, notamment la perte de la diversité génétique au sein des populations et l'homogénéisation -des stocks présumés des affluents en amont du barrage de Mactaquac et la perte d'adaptation aux conditions locales qui en résulte.

Le présent document de recherche rendra compte des résultats des analyses de deux ensembles de données génétiques moléculaires (initialement collectées à d'autres fins), l'un provenant de huit petits échantillons recueillis à l'extérieur de la baie de Fundy, analysé au moyen d'un ensemble limité de 7 locus microsatellites, et l'autre provenant de seulement deux emplacements de l'extérieur de la baie de Fundy, mais analysé au moyen d'un ensemble plus important de 17 locus microsatellites. Les deux ensembles de données ont été collectés dans au moins l'un des affluents du réseau hydrographique du fleuve Saint-Jean en amont du barrage de Mactaquac et dans au moins l'un des affluents en aval du barrage et dans plusieurs populations de référence des autres unités désignables.

Dans l'ensemble, on n'a trouvé que peu de preuves d'une diminution marquée de la diversité génétique de la population dans les affluents en amont du barrage de Mactaquac (potentiellement affectée par des réductions dans le recensement et la taille réelle de la population) par rapport à ceux en aval du barrage de Mactaquac. En effet, les niveaux de diversité dans les échantillons recueillis dans les populations de l'UD de l'extérieur de la baie de Fundy sont dans l'ensemble comparables à ceux recueillis dans les grandes populations de l'UD de la Gaspésie-sud du golfe Saint-Laurent et d'ailleurs, et considérablement plus élevés que pour de nombreux échantillons recueillis dans les populations des UD de l'intérieur de la baie de Fundy et des hautes terres du Sud.

De plus, des différences statistiquement significatives dans les distributions de la fréquence des allèles ont été observées entre deux ensembles d'échantillons recueillis à des endroits situés en amont du barrage de Mactaquac, ce qui laisse à penser que l'accroissement potentiel du flux génétique dû à l'empoissonnement n'a peut-être pas complètement homogénéisé les populations en amont du barrage de Mactaquac. D'un autre côté, les niveaux de structuration génétique des échantillons recueillis dans les affluents en amont du barrage de Mactaquac étaient plus faibles (environ la moitié) que ceux des échantillons recueillis dans les affluents en aval du barrage de Mactaquac.

Bien que ces résultats puissent refléter les effets de l'accroissement du flux génétique dû à l'empoissonnement dans les affluents du réseau hydrographique supérieur du fleuve Saint-Jean, des tendances similaires (différenciation plus importante dans les affluents du fleuve inférieur par rapport aux affluents du fleuve supérieur) ont également été observées dans

d'autres grands réseaux hydrographiques (moins touchés), ce qui indique qu'il est également possible que les tendances dans le réseau du fleuve Saint-Jean puissent refléter des processus biologiques naturels.

Les résultats présentés ici, et des conclusions similaires sur les répercussions de l'empoissonnement sur l'homogénéisation et la dégradation de l'adaptation locale dans d'autres populations de saumons de l'Atlantique en voie de disparition (abordées dans le présent document), indiquent que même si les risques potentiels d'empoissonnement par les écloseries pour les populations sauvages sont considérables, les conclusions sur l'empoissonnement, même à long terme et à grande échelle, impliquant les populations de saumons locales et/ou non locales, ne peuvent pas seules être considérées comme une preuve de l'homogénéisation totale des populations sauvages, de la perte importante de l'adaptation locale et de la valeur adaptative, et que des mesures de conservation visant à protéger les populations restantes ne sont pas justifiées.

INTRODUCTION

Atlantic Salmon populations occupying rivers and streams from the US-Canadian border north to and including the Saint John River (SJR) have long been recognized as being phenotypically distinct from neighbouring salmon of the Inner Bay of Fundy (IBoF) (Perley 1852; Huntsman 1931), and have been collectively referred to as Outer Bay of Fundy (OBoF) Atlantic Salmon. Whereas IBoF salmon appear to exhibit mostly local migration, return after one year at sea, and rely on a high incidence of repeat spawning for population persistence, OBoF salmon are characterized by a large multi-sea winter component, with many older adults migrating to marine waters as far distant as the Labrador Sea and Davis Strait before returning to natal streams to spawn (Ritter 1989). These phenotypic differences parallel geographic patterns of divergence observed at mitochondrial and nuclear genetic marker loci analyzed to date (Verspoor et al. 2002; O'Reilly 2006; Fraser et al. 2010; Vandersteen Tymchuk 2010), indicative of restricted gene flow between the two groups. Substantial genetic differentiation at both mitochondrial and nuclear markers analyzed has also been observed between OBoF Atlantic Salmon and the next geographically proximate group (beyond IBoF salmon) of Canadian Atlantic Salmon populations, those from the Southern Upland (SU) of Southeastern and Eastern Nova Scotia (Verspoor et al. 2005; O'Reilly 2006, Vandersteen Tymchuk 2010; O'Reilly et al. 2012). These findings have led to the recognition of OBoF Atlantic Salmon as an important component of within-species biodiversity (DFO and MNRF 2008), and their identification as a Designatable Unit (DU) by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (COSEWIC 2010). During this same COSEWIC assessment, Atlantic Salmon of the OBoF were assessed as endangered, therefore, a Recovery Potential Assessment (RPA) was conducted to evaluate the feasibility of restoring populations to levels that would ensure a high probability of persistence into the future. The present study was initiated in support of the OBoF RPA (DFO 2014), which Fisheries and Oceans Canada (DFO) undertook in the winter of 2013.

A number of factors have been identified as possibly impacting the feasibility of recovering population abundance in the OBoF DU, including depressed population phenomenon (Clarke et al. 2014; DFO 2014). Under standard population dynamics theory, reductions in density are expected to lessen competition for scarce resources, thereby increasing individual survival, which is then expected to lead to subsequent population growth. Under certain conditions, however, population reductions may also limit individual survival, lower reproductive success, and accelerate rates of population decline (Rose et al. 2001). This positive density dependence, also known as depensation, may result from the reduced ability of individuals to find mates, increased susceptibility to predation, and reduced foraging efficiency (Engen et al. 2003). Depressed population phenomenon may also be associated with a number of possible genetic effects.

The most well-known potential genetic impact of small population size on population persistence is inbreeding, defined as mating between related individuals, or as an increase in genome-wide homozygosity (Allendorf and Luikart 2007). This increasing homozygosity exposes deleterious recessive alleles, which is now generally believed to be the major mechanism responsible for reduced trait performance or inbreeding depression (Wang 2000). As population size and the number of extant families decrease, the likelihood that a given male and female, potential mates, will be related increases. Indeed, inbreeding is probably unavoidable in small closed populations and almost always results in reductions in performance traits in normally outbred species studied (Frankham et al. 2002). Examples of documented inbreeding effects in salmonids resulting from close inbreeding (mating between full or half sibs) include reduced rates of growth in Rainbow Trout (Kincaid 1976a,b; Gjerde et al. 1983), Brook Trout (Cooper 1961), and Atlantic Salmon (Rye and Mao 1998) reared in captivity, and reduced survival of individual Steelhead (Thrower and Hard 2009) and Atlantic Salmon (Ryman 1970) in the wild. Additionally, when population size is restricted for several generations, the number of

common ancestors back in time, or the degree of relatedness between any two potential mates, increases. Looked at another way, when population size is small for an extended period, and as more and more alternate alleles at a given locus are lost through genetic drift, there is an increasing probability that any two alleles passed from a set of parents to their offspring will be identical by descent. In other words, inbreeding, and its associated effects (inbreeding depression), are also expected to accumulate through time in small closed populations. Inbreeding associated with small population size has been shown to heighten susceptibility of populations to disease (Spielman et al. 2004), lower the efficacy of recovery programs based on the release of captive-reared individuals into the wild (Van Oosterhout et al. 2007), and increase the extinction probability of populations managed in captivity (Wright et al. 2008) and in the wild (Saccheri et al. 1998; Vilas et al. 2006).

Another possible immediate genetic effect of small population size on population viability is the loss of local adaptation and fitness resulting from genetic drift. Many traits studied in Atlantic Salmon, for example, size at age (Jonsson et al. 1997), timing of hatching (Donaghy and Verspoor 1997) and age of sexual maturation (Glebe and Saunders 1986), are determined by the combined actions of the environment and genetics. The frequency of alleles influencing traits such as these are determined by four evolutionary forces, drift, selection, mutation, and gene flow (Hartl and Clark 1989). In a large population, allele frequencies are primarily determined by past environmental conditions and the effects of natural selection, but as population size decreases, genetic drift becomes an increasingly important driver of allele frequency changes (Allendorf and Luikart 2007). Indeed, genetic drift has the potential to decrease the frequency of alleles that may be more fit in a given population, and increase others that may actually decrease survival and/or reproductive success at a given point in time.

Small population size may also impact population persistence in the medium to long-term by eroding genetic variation and minimizing the future adaptive potential of Atlantic Salmon. As mentioned above, genetic drift may increase or decrease the frequency of alleles. Severe short-term or moderate but sustained drift may also result in the complete loss of alleles, particularly low-frequency or rare alleles (Waples 1990; Tave 1993) from a given population. Although rare alleles may have been less fit in the recent past (with present-day frequencies reflecting past effects of natural and sexual selection), they may be more fit in future years when cyclic environmental conditions change. For example, a low-frequency variant of the ectodysplasin gene, associated with heavy lateral plating in threespine stickleback (Colosimo et al. 2005), was key to a Lake Washington population's evolutionary response to changing predation regimes between 1957 and 2006 (Kitano et al. 2008). Loss of specific alleles from individual populations may be of particular concern for Maritime Atlantic Salmon because ongoing extirpation of IBoF, OBoF, and SU river populations is creating increasingly large-range disjunctions, decreasing the potential for straying and among-population gene flow to re-introduce alleles lost from any one source.

In addition to the above effects of small population size, the recovery of OBoF Atlantic Salmon may also be impacted by genetic effects of historic and ongoing stocking. Not unlike rivers of Maine (NRC 2004), Nova Scotia (Gibson et al. 2003), and Northern New Brunswick (Caron et al. 2005), rivers of the OBoF have been stocked for many decades, involving transfers of millions of juveniles from various life-history stages (Clarke et al. 2014). Moreover, prior to the 1970s, OBoF rivers were stocked with salmon from multiple locations, including Gulf rivers from Northern New Brunswick (Clarke et al. 2014) separated by over 1,500 km of coastline, and draining into an entirely different basin. Smolt-transplantation studies involving salmon from several Gulf river-populations carried out by Ritter (1975) demonstrated marked reductions in adult return rates in rivers increasingly distant from natal sources, with the lowest return rates of Gulf-origin Atlantic Salmon observed in rivers emptying into the Bay of Fundy. These data, comparable transplantation results from Coho Salmon (Reisenbichler 1988), and the general scale at which local adaptation is generally expected in salmonids (Fraser et al. 2011) suggest

that outbreeding effects (loss of local adaptation) may have been behind reduced return rates in Ritter's study, with possible implications for remaining OBoF Atlantic Salmon. Finally, in the 1960s, construction of multiple dams on the SJR (Figure 1), and concerns over expected cumulative turbine mortality of out-migrating smolts, led to the implementation of an extensive mitigation-stocking program to compensate the stocks above Mactaquac Dam for lost production of Atlantic Salmon. In this stocking program, a portion of adults captured at the Mactaquac fishway (located at the Mactaquac Dam, Figure 1) each year was brought into captivity for artificial spawning (see Clarke et al. 2014 for additional details). Salmon intercepted at Mactaquac Dam likely originated from, and were potentially returning to, specific upper SJR tributaries. The means of identifying the tributary of origin did not exist at that time. As a result, unintentional crosses between salmon from different tributaries were likely carried out, as were crosses between salmon from the same (but unknown) tributary. Offspring from the resulting hybrid or unknown within-tributary crosses were then either:

a) released as juveniles into different tributaries (native or not),
b) released as smolt below Mactaquac Dam, or
c) retained as broodstock for artificial spawning in subsequent years.

Human-mediated and possibly heightened gene flow between tributaries would have occurred primarily through either of the following mechanisms:

a) spawning of hybrid or non-native (non-hybrid) mature male parr released into a given tributary with wild returning female salmon, and
b) spawning of hybrid or non-hybrid returning adults that were originally released into a non-native tributary with native wild returning salmon or wild mature male parr.

Smolt released at or below Mactaquac Dam may also have contributed to gene flow between populations from upper tributaries; although they would not have imprinted on upper tributary waters, salmon do stray, large numbers were released, and stray rates of artificially reared Atlantic Salmon, under some circumstances, may be elevated relative to wild-origin counterparts (Jonsson et al. 1991).

The extent of initial stocking-related introgression of genes from one hypothetical "tributary population" into another would have been determined by many factors, including:

a) the number of hatchery-origin hybrid and non-native mature parr and adult salmon relative to the number of wild native mature parr and adult salmon present at spawning time in a given tributary,
b) the spawning success of non-native, hatchery-origin salmon relative to wild-origin native counterparts in the wild,
c) the existence of phenotypic differences between wild and hatchery-origin salmon on the spawning grounds and the extent of assortative mating that may or may not have taken place,
d) the survival and spawning success of subsequent F1 generation salmon, and
e) the extent of breeding that occurred between F1 generation salmon and the native parental population; in order for introgression to occur, F1 offspring must successfully mate with representatives of the original parental strain or species.

The degree to which any pre-existing genetic structuring will have been eroded, and local adaptation lost, will be dependent on the above (factors a to e) in addition to the strength of selection against non-local and potentially domesticated genotypes (discussed below) in subsequent generations. On the one hand, salmon produced and reared in captivity (and their immediate offspring) often exhibit reduced survival and spawning success in natural river habitat compared to their wild-origin counterparts (Fleming and Petersson 2001; Araki et al. 2009), and wild returning salmon greatly outnumbered hatchery-origin salmon passed above Mactaquac Dam in all but 9 of the last 20 years (Jones et al. 2014). On the other hand, large numbers of

hybrid and non-native (non-tributary specific) offspring were produced and released into the tributaries of the SJR each year for over 40 years, and there were several sources of potential introgression, including via the spawning of hybrid, non-native male parr, which would have been quite numerous in some years, and which would only have had to survive a short period of time before spawning. Indeed, recent research demonstrates that the life-history tactic of males maturing and spawning early in freshwater can accelerate introgression of farm genes into wild, native populations (Garant et al. 2003). It should also be noted, however, that whether or not among-tributary transfers of salmon impacted local adaptation and fitness in subsequent generations also depends on the extent of pre-existing genetic structuring and local adaptation; if there was little or no genetic differentiation among salmon from tributaries before the construction of the Mactaquac Dam, then increasing gene flow among tributaries would be expected to have little genetic effect. Although there is limited direct information about the amount of structuring within the SJR system before construction of the Mactaquac Dam, additional insight can potentially be gleaned from other studies of Atlantic Salmon from large but less impacted river systems (discussed below).

In addition to potentially homogenizing populations, particularly those above Mactaquac Dam, stocking may have had several other negative effects on OBoF populations. First, successful hatchery programs, those that lead to a demographic increase in the number of returning adults, may also increase rates of loss of genetic variation and accumulation of inbreeding, the implications of which were given above. Ryman and Laikre (1991) demonstrated that reducing mortality of a portion of families produced in a given year, those reared in captivity, increases variance in family size across the entire population, which can markedly reduce effective population size, though see Hedrick et al. (2000) for an example of where supplementation of a salmonid population did not reduce effective population size. Finally, extensive recent mitigation stocking may have had a negative genetic effect on wild populations via the adaptation of salmon to captive conditions and relaxation of selection in the wild, collectively referred to as domestication selection (Waples 1999). Recent research indicates that domestication selection, even under supplementation programs intended to mitigate many genetic effects of stocking, can be quite rapid (Araki et al. 2007a, 2007b, 2009; Thériault et al. 2011; Christie et al. 2012; de Mestral et al. 2013), leading to an average decline in fitness as high as 37 percent per generation (Araki et al. 2007b); see also Hess et al. (2012) for an example of where supplementation stocking appeared to have resulted in no detectable effect on the survival or reproductive success of first- and second-generation offspring of captive-reared salmon.

The first objective of this report is to compare levels of within-population genetic diversity among river populations within the OBoF DU, with emphasis on comparisons between salmon populations from tributaries above Mactaquac Dam, potentially more heavily impacted by recent reductions in census population size and stocking-related Ryman-Laikre effects, with those from tributaries below Mactaquac Dam, which may have been less impacted. Analyses of within-population genetic variation is limited to two datasets, both collected for other purposes. The first dataset includes information from 7 microsatellite loci (discussed above) from two tributaries above and two tributaries below Mactaquac Dam, and a second dataset includes information from 17 microsatellite loci, but from only one tributary above and one below Mactaquac Dam. The second objective of this report is to assess levels and patterns of among-population genetic structuring within the OBoF. This analysis is based on the limited set of 7 microsatellite loci currently available for multiple sample locations from this DU, and will focus on the large SJR system, comparing structuring between sample collections from tributaries above Mactaquac Dam (potentially homogenized by past stocking activities) with that from tributaries below Mactaquac Dam (possibly less impacted by recent stocking activities). The third objective is to compare average levels of within-population genetic diversity for the overall OBoF DU (at the larger 17-microsatellite locus dataset) with values reported for Atlantic Salmon populations from other DUs, exhibiting different historic demographic trajectories and general population size.

The fourth and final objective of this report is to assess large-scale patterns of genetic structuring at the 17-locus set of microsatellite loci now available for various rivers within the Maritime Provinces, to further evaluate the original delineation of OBoF Atlantic Salmon DU, which was originally, at least in part, based on fewer and/or less variable genetic markers.

METHODS

SAMPLE COLLECTIONS

Analyses presented here involved comparisons among collections of Atlantic Salmon obtained from different rivers of the OBoF and several other nearby DUs, including IBoF, SU, Eastern Cape Breton (ECB), and Gaspé-Southern Gulf of St. Lawrence (GSGL). River names, river codes, geographic coordinates (latitude and longitude) of the river mouth (or where a given river meets the main stem of a larger river), and respective DUs are given in Table 1. All Atlantic Salmon analyzed were collected from the wild, at the juvenile life-history stage, using electrofishing methods. In most instances, sample collections were obtained in one or two surveys during a single year, but in five instances, samples from an adjacent year were included in order to increase sample size (see Table 1 for details). Microsatellite length variation was assayed across individuals from a given sample collection at a common set of 7 or 17 microsatellite loci (Table 1, see also below). All of the sample collections from the OBoF DU were assayed at the more restricted set of seven loci, including several SJR collections from above, and two from below Mactaquac, but one sample collection from above (TOB00) and one below (NSH00) Mactaquac was also assayed at 17 microsatellite loci. All non-OBoF reference sample collections were assayed at 17 microsatellite loci.

LABORATORY ANALYSES

Fin clips were stored in 1.5 ml microcentrifuge tubes containing 1 ml of ethanol. DNA was extracted and purified using Qiagen's 96-well DNeasy Blood and Tissue kits following the manufacturer's specifications. Polymerase Chain Reaction (PCR) amplifications were carried out for each locus separately in 10 µl volumes containing between 1-100 nanograms of template DNA, 0.2 mM each dNTP, 0.1 µM labelled and unlabelled primers, 1X KCl buffer (10mM Tris HCl, 50 mM KCl, 0.08 percent Nonidet P40), 0.5 units of Taq DNA polymerase supplied by MBI Fermentas and 2.5 mM $MgCl_2$. Primer sequences for loci *Ssa*85, *Ssa*171, *Ssa*197 and *Ssa*202 are given in O'Reilly et al. (1996); SS*sp*2201, SS*sp*2210, SS*sp*2215, SS*sp*2216, SS*sp*1G7 and SS*sp*1605 are given in Paterson et al. (2004); *Ssa*D58, *Ssa*D144, *Ssa*D71, and *Ssa*D486 in King et al. (2005); U3 in Presa and Guyomard (1996); and *Ssos*L417 in Slettan et al. (1995). Primers for the locus *Ssa*D85 are unpublished, but are CTTTGGCTGTTTCAGGTATGAC and CACTGCTCTACAACAGAAGTCTC (T. King, Genbank Accession AF525213). Thermal cycling conditions were as follows: (94°C for 3 min) X1, (94°C for 45 sec, 58°C for 30 sec, 74°C for 1 min)X9, and (94°C for 30 sec, 58°C for 30 sec, and 72°C for 1 min) X 27, followed by a 30-min extension step at 72°C. PCR amplification products were either unpurified (if the locus was being size-fractionated on its own), or combined and purified (e.g., salts and unincorporated dNTP's and non-labeled primers removed) using either Qiagen's 96-well MinElute plates (as specified by the manufacturer) or ethanol precipitation methods. Alleles were size-fractionated using denaturing electrophoresis, and detected using either an MJ Research Base station or an Applied Biosystems 3130XL. Size estimates of alleles from different batches, analyzed on different days and on different platforms, were cross-standardized by including 2 of 10 individuals with known genotypes in each batch of samples analyzed; these two individuals varied across batches, also providing an "internal label" in addition to an external written label as normally used, to identify each set of samples analyzed. Ten samples from each batch of 84 were analyzed twice to identify potential sample placement errors, strip inversions, plate inversions and other laboratory mistakes. Length variation in

samples from given collections was assayed at either all of the above 17 microsatellite loci, or at a restricted set of 7 microsatellite loci, SS*sp*1605, SS*sp*2210, SS*sp*2215, SS*sp*2216, SS*sp*1G7, *Ssa*197 and *Ssa*202.

ESTIMATION OF WITHIN-POPULATION GENETIC VARIATION

Several different measures of within-population genetic variation were estimated for each sample collection and each locus, and values averaged across the relevant set of 7 or 17 microsatellite loci. To permit comparisons of numbers of alleles observed across sample collections of varying size, the standardized number of alleles or *allele richness* (*AR*) was estimated using *FSTAT* version 2.9.3.2 (Goudet 1995; Goudet 2001), which is based on the rarefaction procedure of Hurlbert (1971). In this approach, estimates of the expected number of different alleles for each population are made by repeated sampling of 2N genes, where N is the smallest sample size of diploid genotypes present among the sample collections under study. The observed heterozygosity (ObH) was simply the proportion of all genotypes exhibiting two different alleles. Gene diversity (GD), also referred to as expected heterozygosity, was also estimated using *FSTAT*, and is approximately equal to the likelihood that two alleles randomly drawn from a sample are different. The extent of non-random mating, F_{IS} (*f* from Weir and Cockerham 1984), is approximately equal to (Hs-Ho/Hs), where Hs = GD here, and tests of whether F_{IS} values were significantly different from zero (whether individual loci deviated from Hardy-Weinberg equilibrium (HWE)) were also estimated using *FSTAT*. Tests for linkage disequilibrium (LD), or whether genotypes at one locus were independent of genotypes at another, were carried out for all pairs of loci and for all sample collections, using option 4.2.1 of *Genepop* version 4.0 (Rousset 2008). Wilcoxon signed-rank tests were used to assess whether observed differences in GD and AR between pairs of sample collections were statistically significant. *FSTAT* was used to test for statistical significance of the above measures of within-population diversity between groups of sample collections. Sequential Bonferonni methods (Holm 1979) were used to adjust for multiple comparisons in tests of HWE and LD.

ANALYSES OF LEVELS AND PATTERNS OF AMONG-POPULATION GENETIC VARIATION

Pairwise estimates of F_{ST} (theta from Weir and Cockerham (1984)), and significance of deviations from zero (homogeneity), were estimated for all pairs of sample collections using permutation methods (1000 samples), implemented in the program *Genetix* version 4.02 (Belkhir et al. 2001). The program *Populations* 1.2.28 (2005; written by O. Langella[1]) was used to estimate Nei's pairwise D_A distances between sample collections (Nei et al. 1983), and to construct neighbour-joining (NJ) unrooted phylograms (Saitou and Nei 1987) based on D_A distances. Levels of confidence of phylogenetic groupings were estimated using bootstrapping methods implemented in the program *Populations* 1.2.28, resampling across loci 1,000 times. Output was visualized using the program *TreeView* 1.6.6 (2001; written by R.D.M Page[2]). Factorial correspondence analysis (FCA), adapted for molecular genetic data (She et al. 1987) and implemented here using the program *Genetix* version 4.02, was carried out to visualize the relative genetic similarity of sample collections obtained from different rivers. Phylogenetic and FCA tests were carried out using the maximum number of loci common to all sample collections included in a particular analysis (7 or 17).

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RESULTS

WITHIN-POPULATION GENETIC VARIATION

Measures of within-population genetic diversity varied only modestly across sample collections obtained from within the OBoF DU. Based on the limited seven-locus dataset, GD ranged from 0.816 to 0.851 (0.835 to 0.851 excluding CAN00) (Table 2). Allele richness estimates were also similar, ranging from 9.759 to 11.662 (10.214 to 11.662 again excluding CAN00) (Table 2). The average GD of SJR sample collections above Mactaquac (excluding the more recently obtained TOB05 sample collection) was slightly higher (0.848) than that observed below Mactaquac (0.826), but this difference was not significant (p=0.250). Similarly, average AR of SJR sample collections above Mactaquac (10.872) was slightly higher than that below Mactaquac (10.163), but again this difference was not significant (p=0.285). Both measures of genetic diversity estimated for the sample collections obtained from the very small and nearly extirpated Magaguadavic River population (MAG92 and MAG99) were comparable to the more variable SJR sample collections (Table 2). Differences between sample collections obtained from the same locations a few years apart (TOB00 and TOB05, and MAG92 and MAG99) were modest, with all levels appearing to increase slightly over time (Table 2). The most prominent outlier in this dataset (both in terms of GD and AR) was CAN00, which exhibited moderately reduced levels of both measures relative to all other sample collections analyzed (Table 2).

In many sample collections analyzed at this set of seven loci, GD (also referred to as effective heterozygosity) appeared to differ markedly from ObH (observed heterozgyosity) (Table 2). In some cases, GD was higher, and in other instances lower than ObH, resulting in positive (>0) and negative (<0) F_{IS} values, respectively. Most notably, F_{IS} values of all single loci analyzed in SAV00 were negative, and five were less than -0.05; three single-locus F_{IS} values were significantly less than that expected under HWE conditions (p<0.05). The CAN00 sample collection was also notable in that four of seven loci also exhibited negative F_{IS} values, with three exhibiting values below -0.05; three single-locus F_{IS} values were also significantly less than that expected under HWE conditions (p<0.05). No other single-locus F_{IS} value in this dataset was significantly less than expected under HWE conditions. In most other sample collections, F_{IS} values observed at individual loci were positive, but typically between 0 and 0.05 (Table 2). In any given sample collection, 0, 1 or 2 loci exhibited F_{IS} values significantly greater (p<0.05) than that expected under HWE. Generally speaking, there was minimal linkage disequilibrium between pairs of loci in any given sample collection, with 0-4 pairs out of a possible 21 in any single sample collection deviating from expectations at p<0.05. The single exception to these findings involved the SAV00 sample collection, where 6 of the 19 significant (p<0.05) departures from linkage equilibrium observed in this study were detected.

Contrary to results from the above 7-locus dataset, average GD and AR based on the 17-microsatellite locus dataset were higher in the one sample collection obtained below (NSH00, 0.870 and 16.782, respectively) than the single sample obtained from above Mactaquac (TOB00, 0.851 and 15.923, respectively); differences in GD were significant (p<0.01), differences in AR were not (p=0.061) (Table 3).

Average GD (0.860) and AR (13.453) for OBoF sample collections were similar to comparable values for the GSGL DU (0.866 and 13.883) (Table 4), and no differences were significant (p>0.5). Comparisons were also made between these two DUs based on the seven-locus dataset (data not shown), involving six OBoF sample collections from 1999-2000 (excluding temporal replicates from much earlier or later years), and again both indicators of genetic diversity where higher in the GSGL DU, but only slightly, and differences were not significant (p>0.5). On the other hand, based on the 17-locus dataset, average levels of GD (0.794) and AR (10.152) of IBoF salmon were notably less than values for OBoF salmon (Table 4), and observed differences were significant (p=0.0330 and p=0.0270, respectively). Observed

estimates of GD and AR, based on this same 17-locus dataset, were also greater in OBoF compared to SU salmon, but differences were not significant (Table 4, p=0.1080 and 0.2160, respectively). Comparisons were also carried out between OBoF and both IBoF and SU salmon based on the 7-locus dataset (containing more OBoF sample collections), and yielded similar results (GD and AR were higher in OBoF salmon, data not shown), but only differences in AR (p=0.0120) in the OBoF-IBoF and GD (p=0.0130) in the OBoF-SU comparisons were significant (data not shown). Both average AR and GD were quite similar between the OBoF and ECB sample DUs when estimated based on the 17- (Table 4) and 7-locus datasets (data not shown), and none of the four pairwise comparisons were significant (all p values >0.4).

AMONG-POPULATION GENETIC VARIATION

Pairwise F_{ST} values across the eight OBoF sample collections analyzed at 7 microsatellite loci were modest, ranging from 0.00074 to 0.02571 (Table 5), with an average of 0.0142; this average excludes temporal replicates, sample collections obtained from the same location but in earlier (MAG92) or later (TOB05) years than the majority of sample collections analyzed in this study. This level of among-population differentiation is less than comparable averages for IBoF and SU Atlantic Salmon analyzed at these same seven loci (0.0805 and 0.0519, respectively, data not shown), but similar to values for ECB and GSGL sample collections (0.0165 and 0.0113, respectively, data not shown).

The average pairwise F_{ST} of sample collections obtained above Mactaquac (SAV00 vs SER00, SAV00 vs TOB00, TOB00 vs SER00), largely in the same year, was 0.0089, 0.0098 if only pairwise comparisons between major upper SJR tributaries are considered (SAV00 vs SER00, SAV00 vs TOB00); note, that the Serpentine River is a sub-tributary of the larger Tobique River (Figure 1), itself a tributary of the SJR. However, both these values were lower (less than half) than the comparable pairwise estimate for NSH00 and CAN00 (0.02571) sample collections obtained from lower SJR tributaries. Average pairwise F_{ST} values between the six pairs of sample collections involving an upper and a lower SJR tributary obtained in approximately the same year (2000) was 0.0181, less than that observed between lower SJR tributaries. Pairwise F_{ST} estimates between CAN00 and upper SJR sample collections were consistently greater than pairwise estimates between NSH00 and these same upper SJR sample collections. All pairwise F_{ST} values between sample collections obtained from different SJR tributaries in 1999-2000 were significant, except for the SAV00 and TOB00 comparison (p=12.90). Pairwise F_{ST} values were greater for comparisons involving MAG99 and the more geographically proximate lower SJR tributaries (average = 0.0167) than those involving MAG99 and more distant upper SJR tributaries (average = 0.0061, Table 5). Differences between the only two sample collections obtained from the same major tributary collected in the same year (TOB00 and SER00) were modest (0.00718, Table 5) but significant (p=0.0011). Values of F_{ST} between temporal replicates (TOB00 and TOB05, and MAG92 and MAG99), were small (0.00345 and 0.00074, respectively, Table 5) and non-significant.

Overall, patterns of differentiation among OBoF sample collections discussed above (based on F_{ST} values) for this seven-locus dataset were evident in both the NJ phylogeny (based on Nei's D_A distances) and FCA analyses (figures 2 and 3). Sample collections above Mactaquac clustered together in NJ phylogenetic analysis, as did sample collections from below Mactaquac, and NSH00 and CAN00 were very divergent, as indicated by the long branch lengths in this portion of the phylogeny (Figure 2). In the FCA analyses, samples from above Mactaquac also clustered together, though the greater differentiation between SAV00 and other upper SJR tributaries except TOB00 is evident (SAV00 separates from all other upper sample collections along axis 3, Figure 3). The high degree of differentiation between NSH00 and CAN00 is also apparent in the FCA analysis, though NSH00, not CAN00, is the most divergent from all others, separating mostly along axis 1 (Figure 3). The two sample collections from the Magaguadavic River cluster together in both phylogenetic and FCA analyses (figures 2 and 3),

though MAG92 exhibits the least divergence from other OBoF sample collections in the latter analysis. Bootstrap support for different groupings in the phylogenetic analyses were not included because of the small number of loci available in this dataset.

In the two multi-DU phylogenies based on both the datasets of 7 (containing more OBoF sample collections, Figure 4) and 17 (containing fewer OBoF sample collections, Figure 5) loci, samples from the same DU (identified by like-coloured fonts) generally cluster together, with the exception of GLGS and ECB DU salmon, where many sample collections from the two DUs interdigitate. Other limited exceptions include GRA10, which first clusters with SU sample collections, and ROH00, which first clusters with GAK02 IBoF salmon (figures 4 and 5). Within DUs, sample collections from neighbouring rivers generally cluster together, and then with more distant rivers within the DU. For example, sample collections from Chignecto Bay (USR02, BSR01, PWF02) cluster together, as do collections from the Minas Basin side (GRV01, ECO01, and STW01) of the IBoF (figures 4 and 5). Northeast SU populations (SAG09, COU00, SMA00, and MOS00) group together, as do most Southwest SU populations (SAD00, TSK99, MED01, LAH00 and GLD01), particularly in the NJ phylogeny based on 17 loci (Figure 5). Finally, as mentioned previously, upper SJR tributaries (TOB00, TOB05, SER00 and SAV00) cluster together, as do lower SJR tributaries (CAN00 and NSH00) (Figure 4). Note, once again, bootstrap support for different groupings in the phylogenetic analyses based on seven loci were not included because of the small number of loci used in this analysis.

On a broader scale, the positioning of major branches in the phylogeny representing, for the most part, previously identified DUs generally appears to reflect present-day geography and coastal distance between regions. In other words, geographically neighbouring DUs generally first group together first before clustering with other DUs. For example, IBoF and OBoF DUs cluster together, as do SU and ECB DUs (figures 4 and 5). In an exception to this general pattern, geographically proximate SU and IBoF DUs, are not "neighbours" in either phylogeny (figures 4 and 5). Even more unexpected is the very close specific grouping of RKR03 and the OBoF sample collections (figures 4 and 5), despite the fact that in terms of coastal distance, the associated rivers are the most geographically disparate in the entire study, and are separated by multiple river populations and several DUs.

In the FCA analysis of sample collections obtained from 30 rivers, three major groupings (IBoF, OBoF-ECB-GSGL, and SU) were apparent, mostly separating out on axes 1 and 3; the single sample collection ROH00 was highly divergent from all others, separating on axis 2 (Figure 6). Once again, the greater among-population differentiation was apparent within the IBoF and SU groupings compared to all others (the spatial distance between pairs of IBoF and between pairs of SU sample collections was greater than that observed between most pairs of sample collections from other DUs in this analysis). Also, little differentiation was observed between OBoF, GSGL, and ECB sample collections (Figure 6). However, after removing IBoF and SU salmon, sample collections from OBoF, ECB and GSGL are better resolved: a clear distinction between OBoF and ECB populations is evident, although RKR03 from the GSGL DU again clustered with the two OBoF sample collections analyzed in this study (Figure 7).

DISCUSSION

WITHIN-POPULATION GENETIC VARIATION

Overall, sample collections obtained from the OBoF Atlantic Salmon DU included in this study appear to exhibit relatively high levels of within-population genetic variation compared to salmon in other DUs, somewhat surprising given recent declines in the number of small and large salmon returns to rivers where adult populations are assessed (DFO 2014), and overall low densities of juveniles observed in many rivers surveyed (Jones et al. 2014). Average estimates of both GD and AR based on the 17-locus dataset analyzed here were markedly (and

significantly) higher than those observed in IBoF salmon, moderately higher than SU salmon, slightly higher than ECB salmon, and only marginally lower (within one and approximately three percent, respectively) than those observed in the large GSGL Atlantic Salmon reference populations; similar results, in terms of the relative ranking of DUs and magnitude of differences between DU pairs, were observed in the 7-locus dataset which, although based on fewer loci, included more OBoF sample collections. It could be argued that average levels of genetic diversity in the relatively healthy GSGL DU were slightly reduced by inclusion of MAB06, a sample collection obtained from a smaller river harbouring a modest salmon population, that actually groups with other ECB rivers in phylogenetic analyses and which perhaps should be included along with other geographically proximate Cape Breton populations in the ECB DU (O'Reilly et al. 2013). Levels of both GD and AR are still within three percent if comparisons are instead made between the average for OBoF salmon and the RKR03 sample collection (0.874 and 13.825, respectively), obtained from the Kedgwick tributary of the very large Restigouche River, which saw an estimated 8,859 adult returns in recent years (Caron et al. 2005). Additional insight into how levels of OBoF within-population variation compare to other large healthy rivers may also be gleaned by including information from other studies that also included one or more populations surveyed here. Dionne et al. (2009), in an analysis involving 12 microsatellite loci (all common to this study), also included the Kedgwick in their survey, and reported similar levels of GD (0.88) as observed here for the same tributary. In their study, levels of GD for the Kedgwick River were within two percent of estimates made for five other Restigouche tributaries, and within one percent of the average of tributaries surveyed from the Moisie River (0.87), a large non-stocked system in Quebec which saw an estimated 2,483 salmon in recent years (Caron et al. 2005). The estimate of AR for the Kedgwick in Dionne et al. (2009) (14.0) was nearly identical to that reported here, and similar to the average of all other Restigouche tributaries (13.44) surveyed in their study (in fact, slightly higher), and quite a bit larger than the average for the Moisie River system (12.46). Collectively, these results indicate that average levels of within-population genetic variation in the OBoF do not appear to be much reduced relative to values estimated for several other very large and healthy populations from other DUs.

Interestingly, the rank order of average estimates of GD (GSGL>OBoF>ECB>SU>IBOF) was exactly opposite to the rank order of variance estimates across populations within DUs (GSGL<OBoF<ECB<SU<IBOF). In other words, declines in average levels of within-population genetic variation within a DU were accompanied by increasing among-population heterogeneity in levels of within-population variation; given the direction of observed results, this pattern would be seen with or without standardizing variances to the mean. Both metrics (overall reductions and increasing variance) may be important indicators of the genetic health of a DU. These results also suggest that natural gene flow may be insufficient to maintain levels of variability across river populations within DUs, especially when census population sizes decrease, and populations become increasingly fragmented and isolated. Instead, under such circumstances, levels of within-population variation may be largely determined by intrinsic factors, such as effective population size and the magnitude of genetic drift.

Within the OBoF DU, estimates of genetic diversity varied comparatively little from one sample collection to the next. These results were somewhat surprising given the very different management and demographic histories of OBoF populations surveyed in this study. For example, whereas several hundred adult salmon once returned to the Magaguadavic River yearly, the run began to steadily decline in 1992 (Carr et al. 1997), to the point that fewer than 10 wild returning salmon were intercepted at the Magaguadavic fishway in the years 2000-2008 (Jones et al. 2010). Despite this precipitous and consistent reduction in number of adult returns, GD and AR in the MAG99 collection were very similar to those observed in other contemporary OBoF populations, and similar to the sample collection obtained from the Magaguadavic River (MAG92) the year the decline began, and before impacts of genetic drift associated with the collapse of the wild population on levels of within-population genetic variation would be

expected. The lack of reductions in GD and AR assessed at these same 7 microsatellite loci in additional sample collections spanning 1992-2002 from this same river was also reported by Bourret et al. (2011). Based on further genetic information from hundreds of Single Nucleotide Polymorphism (SNP) loci, and additional analyses carried out in their study, these authors were able to attribute observed results to likely introgression of new alleles from genetically divergent aquaculture salmon. Several freshwater hatcheries on the Magaguadavic River were leaking large numbers of juveniles into system in the 1990s (Stokesbury and Lacroix 1997), and the number of aquaculture-origin adults intercepted at the Magaguadavic fishway, possibly on their way to spawn, in the years 1994-1996, exceeded the number of wild native returning salmon (Carr et al. 1997). These results further suggest that levels of GD and AR observed in the more recent samples collected on the Magaguadavic River likely reflect the combined (and opposing) effects of recent and rapid genetic drift associated with the collapsing wild population, and simultaneous introgression of new genes from aquaculture salmon.

Upper SJR populations of Atlantic Salmon, those upstream of the Mactaquac Dam analyzed here, also experienced very different management regimes and recent demographic trajectories compared to lower SJR populations, specifically, those below Mactaquac surveyed in this study. First, smolt in many upper SJR tributaries, like the Tobique, must pass multiple dams, where cumulative mortality was estimated to be 45 percent (Carr 2001). Atlantic Salmon adults returning to spawn must also pass multiple dams, and associated head ponds, upon returning to spawn. Research on Atlantic Salmon and other salmonids suggests that barriers to upstream fish passage may impact the timing of migration and spawning, spawning site fidelity, spawning success, and survival (reviewed in Thorstad et al. 2008). Hydro dams may also impact the health and size of salmon populations by altering hydrology, temperature, water chemistry, channel morphology, riparian vegetation and general community structure (DFO 2014). Upper SJR populations have also been recipients of extensive recent mitigative stocking, with associated potential impacts, as discussed previously, including possible reductions in genetic diversity via the Ryman-Laikre effect, but also loss of fitness due to the combined effects of domestication selection and loss of local adaptation. Although tributaries below Mactaquac Dam have also been stocked, the impacts were likely reduced, both because local (tributary-specific) broodstock were used in the production of released juveniles (Jones et al. 2010), and because of the scaling back of enhancement activities in recent years (Jones et al. 2014). As a possible result of one or more of the above factors, wild-origin populations above Mactaquac have generally declined previous to, and to a greater extent than, many river populations below Mactaquac, including the Nashwaak River surveyed here (Jones et al. 2014). If effective population size was sufficiently reduced by population size declines, then the above impacts may also have led to increases in rates of loss of genetic variation due to heightened genetic drift. Although this might not at first seem plausible given the number of adults returning to tributaries above Mactaquac (>1000, even in recent years), ratios of census population size to effective number of breeders in wild salmonid populations are often approximately 0.05 to 0.1, and rates of loss of genetic variation are determined by the latter, not the former. Additionally, Ryman-Laikre effects associated with stocking may have further reduced the effective population size, exacerbating rates of loss of genetic variation.

Despite differences in overall management regimes, GD and AR of sample collections obtained from tributaries above and below Mactaquac were quite similar (Table 2), with averages generally within 2 and 6.5 percent, respectively, with levels sometimes higher in sample collections taken above Mactaquac and sometimes higher in sample collections taken below Mactaquac, depending on the set of loci and populations being compared. The only significant difference observed involved GD between the sample collection obtained from the upper SJR Tobique (TOB00) and the lower SJR Nashwaak (NSH00) tributary (with levels higher in the latter), analyzed at a large number (17) of microsatellite loci. It should also be noted, however, that despite relatively high levels of GD and AR observed in the SAV00 collection, all loci

exhibited negative F_{IS} values (several of which were significantly different from zero), and 6 of 21 locus pairs were in linkage disequilibrium (LD). Correlations in gene frequencies among (LD) and within (deviations from HWE) loci are also indicators of population bottlenecks (Wang et al. 1998). Indeed, an excess of heterozygotes can be indicative of very small effective number of breeders, and may be brought about by chance differences in allele frequencies between reproductive males and females (Luikart and Cornuet 1999). Sample collections from Eastern Cape Breton (O'Reilly et al. 2013) and SU (O'Reilly et al. 2012) DUs exhibiting LD and/or an excess of heterozygotes also exhibited either moderate or dramatic reductions in GD and AR. Perhaps the mixed results observed in the SAV00 sample collection (departures from HWE and LD conditions, but seemingly high levels of genetic diversity) reflect the combined effects of population bottlenecks and recent gene flow from other SJR tributaries. Note that in this study, negative F_{IS} values, including several significant departures from zero, were also observed in a single other collection, CAN00, though only one locus pair was in LD. Interestingly, this lower SJR sample collection exhibited the lowest levels of genetic variation (both GD and AR) observed across OBoF sample collections analyzed in this study.

Slight reductions in levels of genetic variation in upper tributary compared to lower tributary populations have also been observed in other large river systems, with little or no impacts of stocking and/or hydro-electric facilities. Primmer et al. (2005) analyzed variation at 17 microsatellite loci in Atlantic Salmon sampled from 11 tributaries of the Varzuga River system of Northern Russia. A negative association between genetic diversity and distance from the river mouth was observed, a likely outcome, according to the authors, of increasing straying of upper tributary salmon into lower river reaches while migrating to natal spawning sites; salmon returning to natal spawning sites in lower tributaries may not pass by upper reaches on their way to spawn, and are therefore thought to be less likely to stray into upper reaches. Similar patterns were evident in Garant et al. (2000) in a five-locus survey of seven sites within the Sainte-Marguerite River, Quebec; upper river sites exhibited lower levels of genetic diversity (GD and AR) compared to lower river sites.

In this study, it is not possible to disentangle the combined effects of possible differential natural straying of upper versus lower tributary salmon, possible increased hatchery-mediated gene flow among tributaries, potential Ryman-Laikre effects on stocked populations, and reductions in census and effective population sizes due to dam- and hatchery-related impacts on current levels of genetic variation observed in upper and lower SJR salmon. However, analyses do indicate that differences between sample collections obtained from upper and lower tributaries are not great in absolute terms (within a few percent) and are relatively low compared to the magnitude of differences observed across sample collections from other DUs (e.g., GD and AR of SU sample collections varied by as much as 20 and 45 percent, respectively (O'Reilly et al. 2012)). Furthermore, levels of within-population genetic variation observed in upper tributary populations are not much reduced relative to the largest and most healthy reference populations from other DUs for which information is available. Finally, differences between upper and lower tributaries are comparable to those seen in other rivers not impacted by dams and much less impacted by recent stocking.

EXTENT AND PATTERNS OF GENETIC STRUCTURING AMONG OBOF ATLANTIC SALMON

Average differentiation between upper SJR tributaries analyzed here, based on contemporary sample collections, was reduced (about half) from that observed between lower SJR tributaries. Indeed, pairwise F_{ST} between upper tributary sample collections SAV00 and TOB00 was minimal (0.00374), not significantly greater than zero, and similar in magnitude to temporal replicates obtained from the same Tobique tributary (0.00345). On the other hand, differences between the SAV00 and SER00 sample collections, obtained from more geographically disparate upper SJR locations, were relatively large for this DU (0.0159) and highly significant,

before or after corrections for multiple tests. In addition, a modest (though significant) degree of differentiation between TOB00 and SER00 (0.00718) was also observed. Although both differences were still small relative to the CAN00-NSH00 comparison (0.02571), several findings suggest caution when interpreting the biological significance and causes of differences in levels of genetic structuring observed between sample collections from above versus below Mactaquac Dam. First, observed differentiation between CAN00 and NSH00 may not be very representative of general levels of structuring below Mactaquac, and may not reflect the duration and degree of long-term reproductive isolation between these two tributaries, thought to be indicative of potential adaptive differences (Waples 1991). Note that the difference in F_{ST} between CAN00 and NSH00 sample collections is considerably greater than that observed between NSH00 and geographically distant upper SJR tributaries, and that differences in F_{ST} between CAN00 and every other SJR sample collection are greater than between NSH00 and these same sample collections. This finding, combined with the observation that GD and AR are quite a bit lower in the CAN00 collection relative to all other OBoF sample collections, suggests that the large amounts of divergence between CAN00 and NSH00 may, at least in part, reflect high levels of recent rapid genetic drift in the Canaan population. Although little information is available on the size of the adult population in the Canaan, recent assessments of juvenile densities in these two rivers were quite similar (Jones et al. 2014). Second, levels of differentiation between sample collections from upper reaches compared to differentiation between sample collections from lower reaches in other large river systems, subjected to little or no recent stocking, can also be quite variable, due presumably to the action of divergent natural biological processes in these rivers. Whereas pairwise F_{ST} values between sample collections obtained from upper sites, and those between sample collections from lower sites, were similar in Varzuga River (Primmer et al. 2005), levels of differentiation were higher between upper tributary collections compared to lower tributary collections on the Sainte-Marguerite (Garant et al. 2000), but lower in analogous comparisons for the Restigouche river (Dionne et al. 2008). Additionally, sample-based estimates of true population values carried out here may be imprecise (discussed further below). Finally, the geographic distance between Tobique and Salmon River (Victoria) river mouths is much reduced relative to the distance between Nashwaak and Canann river mouths, and this may impact natural stray rates and gene flow.

Based on analyses of a limited set of 7 microsatellite markers and relatively small sample sizes, average pairwise F_{ST} values were modest across the OBoF DU, markedly less than the average observed in IBoF and SU DUs, but similar to ECB and GSGL DUs. Reduced differentiation across OBoF (as well as ECB and GSGF sample collections) compared to IBoF and SU sample collections is also readily apparent in both NJ phylogenies as shorter branch lengths in clusters involving OBoF salmon, and in FCA analyses as reduced spatial separation between OBoF populations (figures 4, 5, and 6), compared to IBoF and SU populations. Observed variability in average levels of differentiation across Maritime DUs has several possible sources. First, many OBoF comparisons included upper SJR salmon; extensive and recent stocking above Mactaquac may have, to some extent, minimized differentiation between these collections, reducing the average pairwise distances across the OBoF DU (see above). Second, OBoF sample collections analyzed here were mostly restricted to a single large river (the SJR), whereas sample collections from other DUs were obtained in entirely different rivers, each emptying independently into the Atlantic Ocean. Stray rates, and potentially associated gene flow, which would tend to limit differentiation between populations, may be higher between tributaries of even large river systems compared to rates between smaller independent rivers. However, it is interesting to note that average pairwise F_{ST} values between either the historic (MAG92) or more contemporary (MAG99) sample collections from the Magaguadavic River, and sample collections from the SJR, were also modest, averaging 0.00781 and 0.00926, respectively. In other words, reduced differentiation observed here may not be entirely driven by differences in sample collection regimes (within larger river systems versus among small independent rivers) across the different DUs. It should be noted, however, that low levels of

differentiation between these two rivers may also reflect the colonization and stocking history of the Magaguadavic River; the Magaguadavic population may have been introduced via stocking of SJR salmon (Marshall et al. 2014), though it is also possible that stocking had little effect and that the river was colonized via natural straying of salmon from neighbouring rivers in the US and Canada. Third, variation in levels of differentiation observed across Maritime DUs may reflect recent population reductions, decreases in effective population size, and increased effects of recent genetic drift. If so, then one might expect to see a negative relationship between measures of average within-population genetic variation and average among-population differentiation across DUs. Indeed, both AR and GD are highly negatively correlated with F_{ST} (r=-0.9878, p=0.002, and r=-0.9767, p=0.004, respectively) and standardized F_{ST} (G'_{ST}) (r=-0.9488, p=0.014, and r=-0.9734, p=0.005).

FURTHER EVALUATION OF THE ORIGINAL DELINEATION OF THE OBOF DU

Analyses of microsatellite genetic variation carried out here are largely concordant with the identification of OBoF salmon as an important component of within-species biodiversity, and their resolution as a DU of Atlantic Salmon. In the seven-locus NJ phylogenetic analysis, all eight OBoF sample collections cluster together and separate from all others; although the 17-locus phylogeny includes only two OBoF sample collections, these also cluster together, with strong bootstrap support (94 percent). Additionally, the remaining branching patterns in the two phylogenies are very similar. Although OBoF salmon also cluster with ECB and GSGL populations in the large-scale FCA analyses, a clear distinction between OBoF and most ECB and GSGL populations becomes apparent when SU and IBoF salmon are removed from the analyses. The one exception is the continued close association between the single GSGL RKR03 collection and the OBoF group in both the FCA (Figure 7) and phylogenetic analyses, including (in particular) that based on 17 loci (Figure 6), where the RKR03-OBoF clade receives 86 percent bootstrap support. Today the upper tributaries of the Restigouche and Tobique rivers nearly interdigitate in Northern New Brunswick. Perhaps these systems were even more closely associated in the past, allowing movement of individuals between rivers via a possible historic freshwater corridor. Alternatively, observed similarity between these two groups may reflect historic coastlines and patterns of post-glacial colonization, and/or historic gene flow mediated by straying of returning adults into adjacent rivers. As a result of the combined effects of sequestration of ocean water in continental ice sheets the world over, and depression of the North American lithosphere by the massive Laurentide ice sheet, the coastline of the Bay of Fundy area has been quite variable throughout the Quaternary, with shorelines ranging from 70 metres above to 100 metres below present levels (Stea et al. 2001). Maximum elevation between northeast portions of the Bay of Fundy and the Northumberland Strait near Jackson's Point (along the NS/NB border) is currently less than 30 metres. Coastal distances between historic Bay of Fundy and New Brunswick Gulf river mouths may have been much less in the past, possibly at some point in the Quaternary period when river habitat was accessible to colonizing Atlantic Salmon. More research is needed to further explore patterns of genetic differentiation at other molecular genetic markers (e.g., mitochondrial and nuclear Single Nucleotide Polymorphisms) in these groups of Atlantic Salmon (and other anadromous fishes) across this same geographic distribution in the context of historic colonization and gene flow.

Finally, ecological variation across Maritime rivers may also be contributing to patterns of genetic variation observed here, including the similarity noted between OBoF and Northern Gulf salmon. Dionne et al. (2008) found that river temperature regime was associated with the pattern of genetic structuring (at neutral microsatellite loci) observed across Quebec and Labrador populations of Atlantic Salmon surveyed in their study. Several lines of evidence cited by these authors, including positive associations between genetic diversity at the major histocompatibility complex (MHC) class IIB gene and temperature in these same populations of Atlantic Salmon (Dionne et al. 2007), suggest that local adaptation to thermal regimes (and

other associated ecological conditions) may be constraining gene flow among some populations in their study. If the geographic scale of straying is sufficiently broad, then a similar mechanism may have contributed to patterns observed here; offspring of strays from larger Gulf populations may have exhibited high survival in ecologically similar OBoF rivers (compared to other Maritime rivers) increasing gene flow and reducing the potential for differentiation to accrue via genetic drift. Dionne et al. (2008) did indeed report a similar proportion of migrants within versus between regional groups of Atlantic Salmon analyzed in their study. On the other hand, as discussed above, return rates of Gulf-origin smolts released into rivers draining into the Bay of Fundy were markedly reduced relative to those reported for smolts from these same populations released into non-native Gulf rivers (Ritter 1975), suggesting a 1,000-plus kilometre scale of local adaption (Fraser et al. 2011), presumably (given the life stage involved) associated with marine conditions. It is possible that loss of fitness of offspring of Gulf-origin parents produced in OBoF rivers associated with lower marine survival was more than offset by higher survival in OBoF freshwater environments (potentially similar to New Brunswick Gulf river environments), at least compared to the combined freshwater and marine fitness of offspring of Gulf strays in other Maritime rivers (e.g., SU rivers) where native populations exhibit higher levels of genetic divergence from Northern Gulf salmon (e.g., SU Atlantic Salmon, Figure 7).

SUMMARY AND CONCLUSIONS

Despite concerns over possible effects of recent population declines and extensive mitigation stocking on effective population size and rates of genetic drift experienced by river populations above Mactaquac, sample collections obtained in upper SJR tributaries, particularly those from the Tobique and Serpentine tributaries, appear to be moderately variable, perhaps slightly reduced relative to some sample collections from tributaries below Mactaquac Dam and sample collections obtained from other large river systems harbouring large healthy populations of Atlantic Salmon. In other words, salmon populations surveyed here from above Mactaquac do not appear to be genetically depauperate and, with the possible exception of the Salmon River (Victoria) population, may not have experienced the same severe population bottlenecks as have some SU and IBoF populations.

We also see some evidence for statistically significant genetic differentiation among sample collections obtained above Mactaquac (e.g., SAV00 versus SER00, SER00 versus TOB00), with the former exhibiting levels of differentiation comparable to averages observed among populations within the ECB and GSGL DUs, both potentially less impacted by recent stocking. These results suggest that possibly heightened, stocking-mediated gene flow may not have completely homogenized upper SJR populations, although levels of genetic structuring may be reduced in upper relative to lower SJR populations. However, there are a number of potential caveats associated with these findings. First, the differences observed between some sample collections (SAV00 and TOB00, and TOB00 and SER00) were small, similar in magnitude to that observed between sample collections obtained from the same location in different years. Certain characteristics of Atlantic Salmon biology, and the nature of sample collections possibly obtained here, can confound results, particularly when observed levels of differentiation are of this magnitude. For example, in species with overlapping generations such as Atlantic Salmon, some genetic differentiation between cohorts can be expected (NRC 2002). Varied representation of different year classes in spatial samples (e.g., samples from different tributaries) can thus lead to overestimates of differences between river populations. Additionally, limited dispersal of fry and parr from redds, combined with geographically restricted sampling, can result in collections of salmon comprising relatively few kin groups, and sample collections that poorly reflect the actual or true population. In other words, the presence of family structuring (multiple half- or full-sib groups) within sample collections can inflate estimates of between-population differences, and result in inappropriate findings of statistically significant differences between populations that may indeed be homogeneous (Allendorf and Phelps 1981).

Undetected kinship can also lead to departures from HWE, and inappropriate inferences of potential population-level effects on measures of within-population variation (Castric et al. 2002). Furthermore, sample sizes of collections analyzed here were very small (as low as 26), impacting the accuracy of estimates of both within- and among-population genetic variation. Indeed, error in estimating pairwise F_{ST} is 1/2N, where N is the sample size obtained from a given location (Waples 1998). Given the shallow structuring observed here, this is particularly noteworthy. Finally, many comparisons within the SJR were based on only 7 microsatellite loci, a very restricted sampling of the Atlantic Salmon genome; recent analyses of within- and among-population variation in Atlantic Salmon typically involve 12 or more loci (King et al. 2001; Primmer et al. 2005; Dionne et al. 2008; O'Reilly et al. 2012, 2013).

Given the above considerations, these results provide evidence (though limited) for the presence of genetic structuring among OBoF populations of Atlantic Salmon, including those above Mactaquac Dam. It should be understood, however, that even if the above sample collection limitations did not exist, one could still not conclude that differences observed reflected long-term reproductive isolation versus either:

a) the action of higher levels of genetic drift operating over the course of one or a few decades, or
b) a combination of other potentially opposing anthropogenic factors, including increased rates of hatchery-mediated gene flow combined with very high rates of genetic drift due to Ryman-Laikre effects and recent reductions in census and effective population sizes.

Some of these same limitations, including the inability to distinguish between the relative roles of rapid recent drift versus long-term reproductive isolation, has also been noted by NRC (2002) for recent findings of genetic differentiation observed among river-populations of Maine Atlantic Salmon (King et al. 2000, 2001; Spidle et al. 2003). Despite the fact Maine Atlantic Salmon have been stocked for over 130 years, involving release of 120 million juveniles of various life stages from several local and non-local sources (NRC 2004), recent and extensive re-analyses and re-interpretation of the above cited molecular genetic data (and other genetic information) by the National Research Council lead to the conclusion that individual river populations in the state of Maine "are not mainly hatchery mixtures" (NRC 2002). The rationale for these conclusions includes observations that levels of genetic structuring observed in Maine were similar to that reported for more natural (less impacted) rivers in North America, and findings by Fleming and Petersson (2001) that in 14 of 31 studies of the effects of hatchery releases on wild populations of Pacific and Atlantic Salmon, little or no evidence of introgression of hatchery genes into wild populations was detected, despite the long-term nature of the hatchery releases. In other words, hatchery releases, particularly those from other sources, may have been ineffective, failing to return, and failing to successfully reproduce at moderate or high frequencies. These same arguments would appear to apply to upper SJR salmon which, in at least one comparison, exhibit similar levels of genetic structuring to that observed between larger less impacted populations from the GSGL, and comparable stocking histories (in terms of numbers of individuals, life stages released, and native versus non-native origin of broodstock used) to Maine rivers. Clearly, although the potential risks of hatchery stocking on wild populations are significant, even large-scale, long-term stocking, involving either local or non-local salmon, does not necessarily indicate that homogenization of wild populations has taken place, that extensive loss of local adaptation and fitness has occurred, and that conservation measures aimed at protecting remaining populations are not warranted.

ACKNOWLEDGEMENTS

We thank Kris Gordos, Andrea Cox, Louise de Mestral Bezanson, Manon Cassista Da-Ros, and Roxanne Gillett for assisting with laboratory analyses. Leroy Anderson, Jonathan Carr, Jamie Gibson, and Beth Lenentine contributed samples analyzed in this study. The authors are also indebted to Carolyn Harvie, Shane O'Neil, and Lorraine Hamilton for reviewing copies of this document. Funding for this research was provided by DFO's Species at Risk program, and the Canadian Regulatory System for Biotechnology.

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TABLES

Table 1. Sample collection information, including geographic location, year(s) of collection, and associated microsatellite locus set(s).

Sample collection name (river)	Sample code	Year(s) of collection	Latitude(N), Longitude (W)	Salmon Fishing Area (SFA)	DU [unit number]	Sub-region	Locus set (17L/7L)	Sample size*
Salmon, Victoria	SAV00	2000	46-56-54,67-39-02	23	OBoF [16]	Saint John R, above	7	26
Serpentine	SER00	2000	47-14-37,66-59-29	23	OBoF [16]	Saint John R, above	7	40
Tobique[1]	TOB00	2000(2001)	46-45-59,67-42-04	23	OBoF [16]	Saint John R, above	17/7	84/46*
Tobique	TOB05	2005	46-45-59,67-42-04	23	OBoF [16]	Saint John R, above	7	73
Nashwaak	NSH00	2000	45-57-27,66-37-19	23	OBoF [16]	Saint John R, below	17/7	70/43
Canaan	CAN00	2000	45-52-56, 65-50-27	23	OBoF [16]	Saint John R, below	7	30
Magaguadavic	MAG92	1992	45-06-55,66-52-56	23	OBoF [16]	Non-Saint John R	7	48
Magaguadavic[2]	MAG99	1999(2000)	45-06-55,66-52-56	23	OBoF [16]	Non-Saint John R	7	50
Big Salmon	BSR01	2001	45-30-53,65-09-34	22	IBoF [15]	Chignecto Bay	17/7	81
Point Wolfe	PWF02	2002	45-32-58,65-01-02	22	IBoF [15]	Chignecto Bay	17/7	46
Upper Salmon[3]	USR02	2002(2001)	45-35-57,64-56-54	22	IBoF [15]	Chignecto Bay	17/7	54
Economy	ECO01	2001	45-22-41,63-54-45	22	IBoF [15]	Minas Basin	17/7	30
Great Village	GRV01	2001	45-23-41,63-36-25	22	IBoF [15]	Minas Basin	17/7	37
Stewiacke	STW01	2001	45-08-28,63-22-43	22	IBoF [15]	Minas Basin	17/7	82
Gaspereau	GAK02	2002	45-06-02,64-16-10	22	IBoF [15]	Minas Basin	17/7	66
Round Hill	ROH00	2000	44-46-21,65-25-41	21	SU [14]	Southwest SU	17/7	28
Salmon, Digby	SAD00	2000	44-03-09,66-09-41	21	SU [14]	Southwest SU	17/7	44
Tusket	TSK99	1999	43-51-46,65-58-48	21	SU [14]	Southwest SU	17/7	60
Medway	MED01	2001	44-08-05,64-37-34	21	SU [14]	Southwest SU	17/7	83
Lahave	LAH00	2000	44-22-12,64-30-04	21	SU [14]	Southwest SU	17/7	49
Gold	GLD01	2001	44-33-07,64-19-22	22	SU [14]	Southwest SU	17/7	84
Musquodoboit	MSQ00	2000	44-47-26,63-08-14	22	SU [14]	Northeast SU	17/7	53
Moser	MOS00	2000	44-58-23,62-15-16	22	SU [14]	Northeast SU	17/7	58
Saint Mary's	SMA00	2000	45-08-17,61-59-02	22	SU [14]	Northeast SU	17/7	78
Country Harbour	COU00	2000	45-09-31,61-41-09	22	SU [14]	Northeast SU	17/7	42
Salmon River, Guysborough	SAG09	2009	45-21-06,61-30-22	22	SU [14]	Northeast SU	17/7	30
Inhabitants	INH10	2010	45-36-01,61-13-31	19	ECB [13]	ECCB	17/7	53
Grand	GRA10	2010	45-38-49,60-39-44	19	ECB [13]	ECCB	17/7	53
Indian (Eskasoni)[4]	IND07	2007(2006)	45-56-32,60-36-10	19	ECB [13]	BL	17/7	52
Middle, Victoria	MDV06	2006	46-04-59,60-54-30	19	ECB [13]	NCCB	17/7	73
Baddeck	BAD10	2010	46-06-00,60-50-20	19	ECB [13]	NCCB	17/7	52
North, Victoria	NRV06	2006	46-18-10,60-37-11	19	ECB [13]	NCCN	17/7	73
North Aspy[5]	NRA07	2007(2006)	45-54-18,60-30-45	19	ECB [13]	NCCB	17/7	44
Margaree	MRG01	2001	46-04-06,61-22-56	18	GSGL [12]	WCCB	17/7	49
Mabou	MAB06	2006	46-25-59,61-06-00	18	GSGL [12]	WCCB	17/7	80
Kedgwick	RKR03	2003	47-39-55,67-29-34	18	GSGL [12]	WGSL	17/7	58

Note: OBoF-Outer Bay of Fundy; IBoF-Inner Bay of Fundy; SU-Southern Upland; ECB-Eastern Cape Breton; GSGL-Gaspé-Southern Gulf of St. Lawrence; Saint John R, above=Saint John River above Mactaquac Dam; Saint John R below=Saint John River below Mactaquac Dam; ECCB=East Coast Cape Breton; BL=Bras d'Or Lakes; NCCB=North Central Cape Breton; WCCB=West Coast Cape Breton; WGSL=Western Gulf of Saint Lawrence; 17L=17-locus dataset; 7L=7-locus dataset; *N for 17-locus dataset above, N for 7-locus dataset below diagonal (only one value given if N is identical for 7- and 17-locus datasets; 1-34 of 84 samples obtained from 2000, 50 from 2001 for 17-locus dataset, all 46 samples from 7-locus dataset obtained in 2000; 2-21 of 50 samples obtained from 2000, 29 from 1999; 3-24 of 54 samples obtained from 2001, 30 from 2002; 4-12 of 52 samples obtained from 2006, 40 from 2007; 5-15 of 44 samples obtained from 2006, 29 from 2007.

Table 2. Within-population genetic variation for eight OBoF sample collections based on 7 microsatellite loci.

Statistic	SAV00 (Above Mac.)	TOB00 (Above Mac.)	TOB05 (Above Mac.)	SER00 (Above Mac.)	NSH00 (Below Mac.)	CAN00 (Below Mac.)	MAG92 (Non-SJR.)	MAG99 (Non-SJR.)
GD (Average)	0.845	0.850	0.851	0.848	0.835	0.816	0.848	0.851
GD (Variance)	0.009	0.006	0.010	0.006	0.008	0.009	0.006	0.007
ObH (Average)	0.900	0.845	0.820	0.811	0.807	0.829	0.801	0.817
ObH (Variance)	0.013	0.008	0.008	0.017	0.003	0.022	0.008	0.021
AR(25) (Average)	10.214	11.295	11.662	11.107	10.566	9.759	10.880	11.274
AR(25) (Variance)	10.586	13.473	13.629	7.452	6.620	12.592	9.854	13.056
F_{IS} (Average)	-0.070	0.004	0.030	0.036	0.008	-0.010	0.030	0.023
F_{IS} (Variance)	0.001	0.002	0.003	0.006	0.006	0.006	0.002	0.007
F_{IS} <0	7	2	2	3	2	4	2	4
F_{IS} <-0.05	5	1	0	0	2	3	0	0
F_{IS} >0	0	5	5	4	5	3	5	3
F_{IS} >0.05	0	1	2	2	2	2	3	1
LD (p<0.05)	6	4	3	2	2	1	0	1
LD (p<0.01)	1	0	2	0	0	0	0	0

Note: GD = gene diversity; ObH = observed heterozygosity; AR(25) = allele richness standardized to 25 diploid individuals; F_{IS} <0 = number of single-locus F_{IS} values less than 0; F_{IS} <-0.05 = number of single-locus F_{IS} values less than -0.05; F_{IS} >0 = number of single-locus F_{IS} values greater than 0; F_{IS} >0.05 = number of single-locus F_{IS} values greater than 0.05. LD (p<0.05) = number of pairs of loci that deviate from linkage equilibrium at alpha 0.05 or less; LD (p<0.01) = number of pairs of loci that deviate from linkage equilibrium at alpha 0.01 or less; Above Mac. indicates sample collection obtained from above the Mactaquac Dam; Below Mac. indicates sample collection obtained from below the Mactaquac Dam; and Non-SJR. indicates sample obtained from a source other than the Saint John River. Full sample names corresponding to alphanumeric codes are given in Table 1.

Table 3. Estimates of within-population genetic variation for Tobique and Nashwaak sample collections based on 17 microsatellite loci.

	Gene diversity			Allele richness		
Locus	TOB00	NSH00	Direction of change	TOB00	NSH00	Direction of change
*SSsp*1605	0.848	0.860	+	10.899	11.657	+
*SSsp*2201	0.951	0.950	-	27.532	26.094	-
*SSsp*2210	0.695	0.686	-	7.372	6.943	-
*SSsp*2215	0.871	0.868	-	11.868	12.453	+
*SSsp*2216	0.920	0.933	+	20.102	21.280	+
*SSsp*1G7	0.863	0.907	+	13.743	12.832	-
*Ssa*197	0.864	0.892	+	11.563	14.623	+
*Ssa*202	0.868	0.854	-	12.881	13.904	+
*Ssa*D486	0.634	0.753	+	8.323	7.995	-
*Ssa*D144	0.930	0.935	+	23.539	23.438	-
SsaD71	0.927	0.943	+	24.168	24.206	+
Ssa171	0.921	0.926	+	19.102	25.094	+
*Ssa*D58	0.934	0.952	+	25.608	26.000	+
*Ssa*85	0.704	0.768	+	7.286	8.915	+
U3	0.698	0.716	+	4.905	6.653	+
*Ssos*L417	0.892	0.889	-	16.829	14.305	-
*Ssa*D85	0.947	0.956	+	24.978	28.897	+
Average	0.851	0.870		15.923	16.782	
Variance	0.010	0.008		54.157	57.837	

Note: Direction of change denotes whether there was an increase (+) or decrease (-) in genetic variation from TOB00 to NSH00 sample collection at individual loci. Full sample names corresponding to alphanumeric codes are given in Table 1.

Table 4. Within-population genetic variation for sample collections from the OBoF and reference DUs based on 17 microsatellite loci.

DU	Sample collection		GD	AR(25)
	TOB00		0.851	13.068
OBoF	NSH00		0.870	13.838
		Average	**0.860**	**13.453**
		Variance	0.000	0.297
IBoF	BSR01		0.827	11.512
	PWF02		0.810	10.682
	USR0102		0.800	10.032
	ECO01		0.700	6.652
	GRV01		0.811	10.947
	STW01		0.808	11.143
	GAK02		0.803	10.100
		Average	**0.794**	**10.152**
		Variance	0.002	2.669
Southern Upland	ROH00		0.676	7.392
	SAD00		0.800	11.511
	TSK99		0.838	11.619
	MED01		0.829	13.044
	LAH00		0.822	11.971
	GLD01		0.833	12.042
	MSQ00		0.841	11.632
	MOS00		0.808	11.899
	SMA00		0.836	13.187
	COU00		0.845	12.092
	SAG09		0.832	13.323
		Average	**0.814**	**11.792**
		Variance	0.002	2.555
ECB	INH10		0.830	12.367
	GRA10		0.852	13.086
	ESK0607		0.840	12.181
	MDV06		0.845	14.007
	BAD10		0.845	13.612
	NRV06		0.827	13.143
	NRA0607		0.847	13.676
		Average	**0.841**	**13.153**
		Variance	0.000	0.463702
Gaspé-Southern Gulf of St. Lawrence	MRG01		0.870	14.355
	MAB06		0.853	13.469
	RKR03		0.874	13.825
		Average	**0.866**	**13.883**
		Variance	0.000	0.198633

Note: GD = gene diversity; AR(25) = allele richness standardized to 25 diploid individuals. Full sample names corresponding to alphanumeric codes are given in Table 1.

Table 5. Pairwise F_{ST} estimates (above diagonal) of between-sample collections obtained from the OBoF in the years 1992-2005 based on 7 microsatellite loci.

Sample collection	SAV00	SER00	TOB00	TOB05	NSH00	CAN00	MAG92	MAG99
SAV00	***	0.01590	0.00374	0.01111	0.01362	0.02187	0.01142	0.00943
SER00	0.0000	***	0.00718	0.00104	0.01796	0.01797	0.00458	0.00742
TOB00	0.1290	0.0011	***	0.00345	0.01651	0.02076	0.00395	0.00147
TOB05	0.0000	0.296	0.0550	***	0.01605	0.02136	0.00257	0.00385
NSH00	0.0010	0.0000	0.0000	0.0000	***	0.02571	0.01317	0.01888
CAN00	0.0000	0.0000	0.0000	0.0000	0.0000	***	0.01119	0.01455
MAG92	0.0030	0.0550	0.0610	0.0920	0.0000	0.0010	***	0.00074
MAG99	0.0050	0.0012	0.231	0.0260	0.0000	0.0010	0.3560	***

Note: Numbers below diagonal reflect the statistical significance of associated pairwise comparisons above the diagonal.

FIGURES

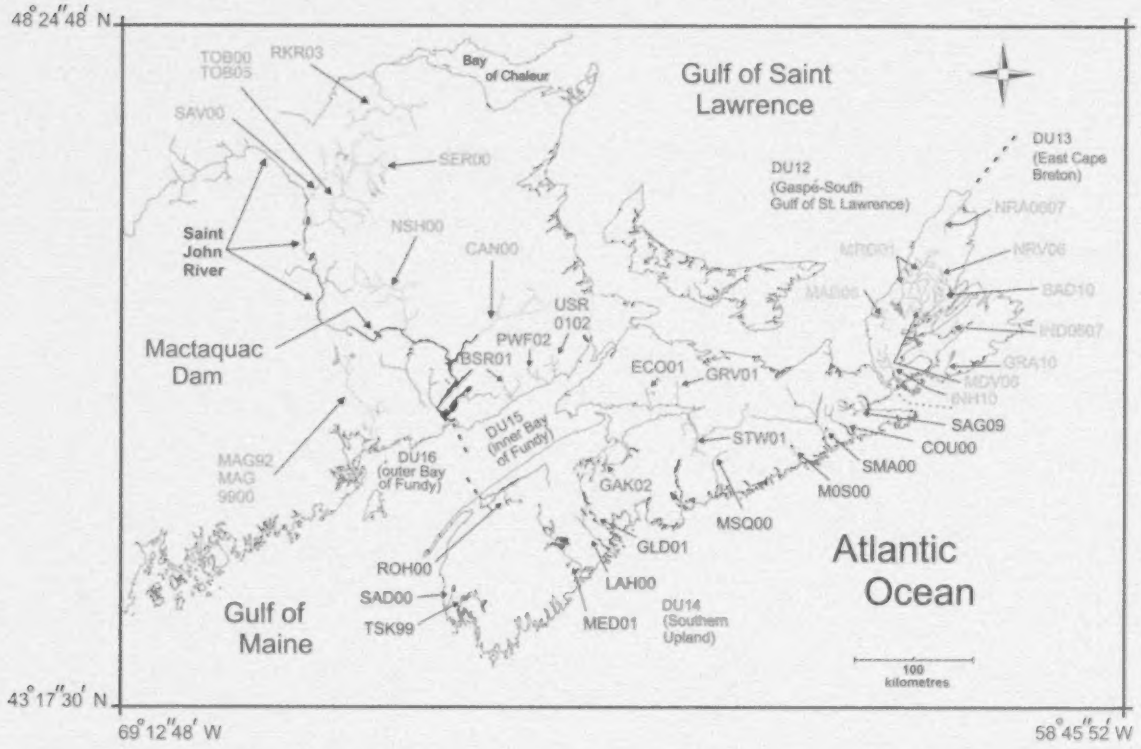

Figure 1. Geographic location of sampled rivers from the OBoF DU (orange), and reference rivers from the nearby IBoF (red), Southern Upland (purple), Eastern Cape Breton (green) and Gaspé-Southern Gulf of St. Lawrence (blue) Designatable Units. Full sample names corresponding to alphanumeric codes are given in Table 1.

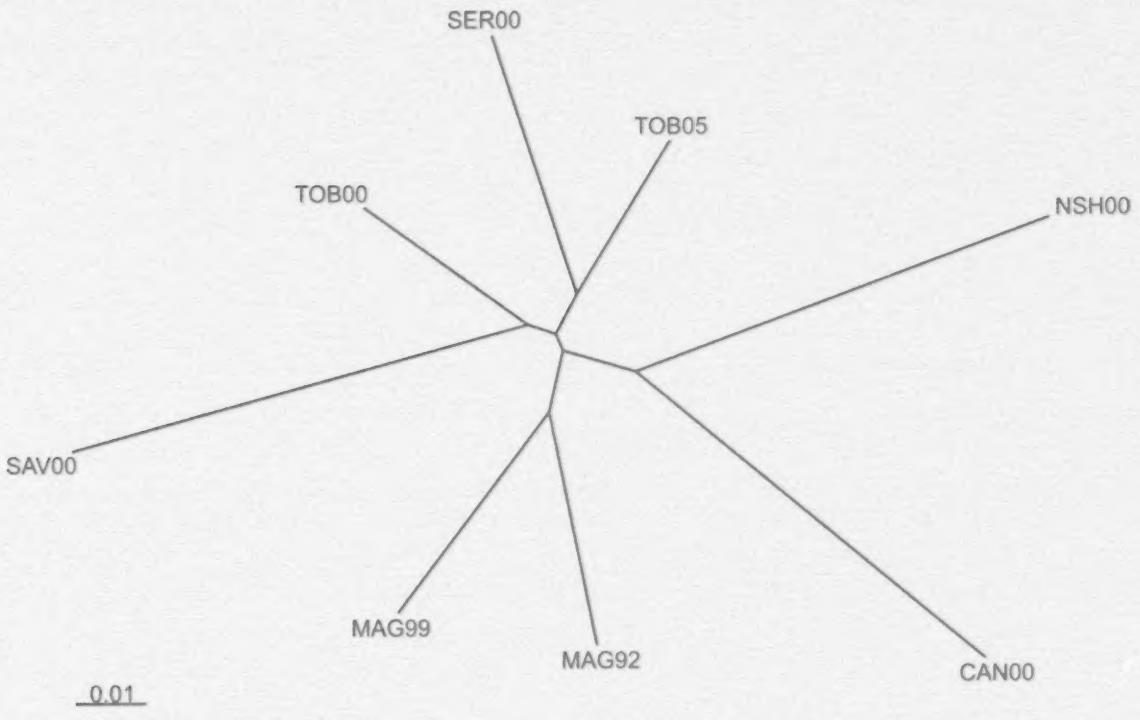

Figure 2. Phylogeny of OBoF sample collections based on Nei's D_A pairwise genetic distances estimated from 7 microsatellite loci, constructed using the neighbour-joining method. Full sample names corresponding to alphanumeric sample codes are given in Table 1.

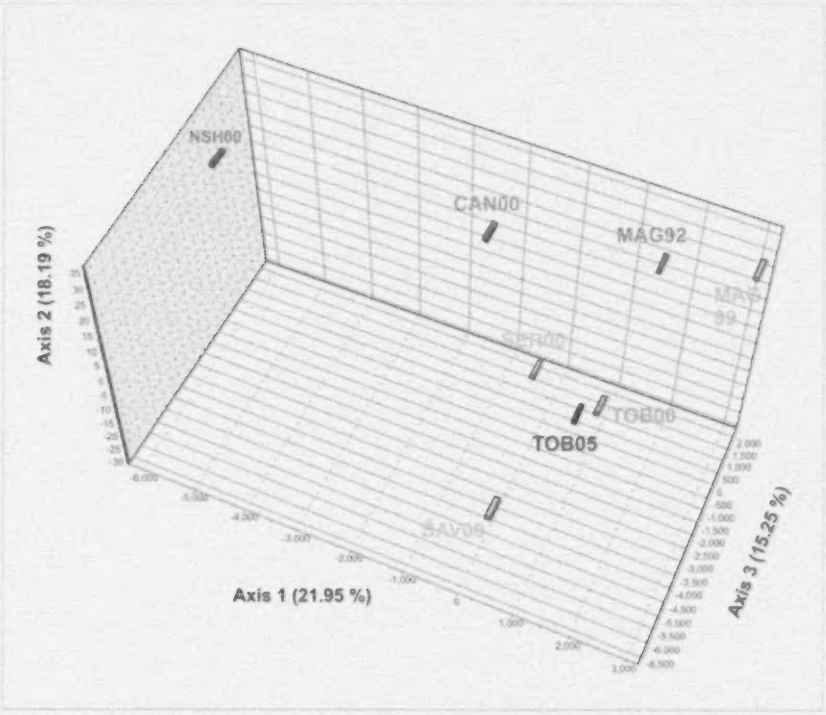

Figure 3. Factorial correspondence analysis of genotype information obtained from 7 microsatellite loci for all eight sample collections obtained from the OBoF DU. Full sample names corresponding to alphanumeric sample codes are given in Table 1.

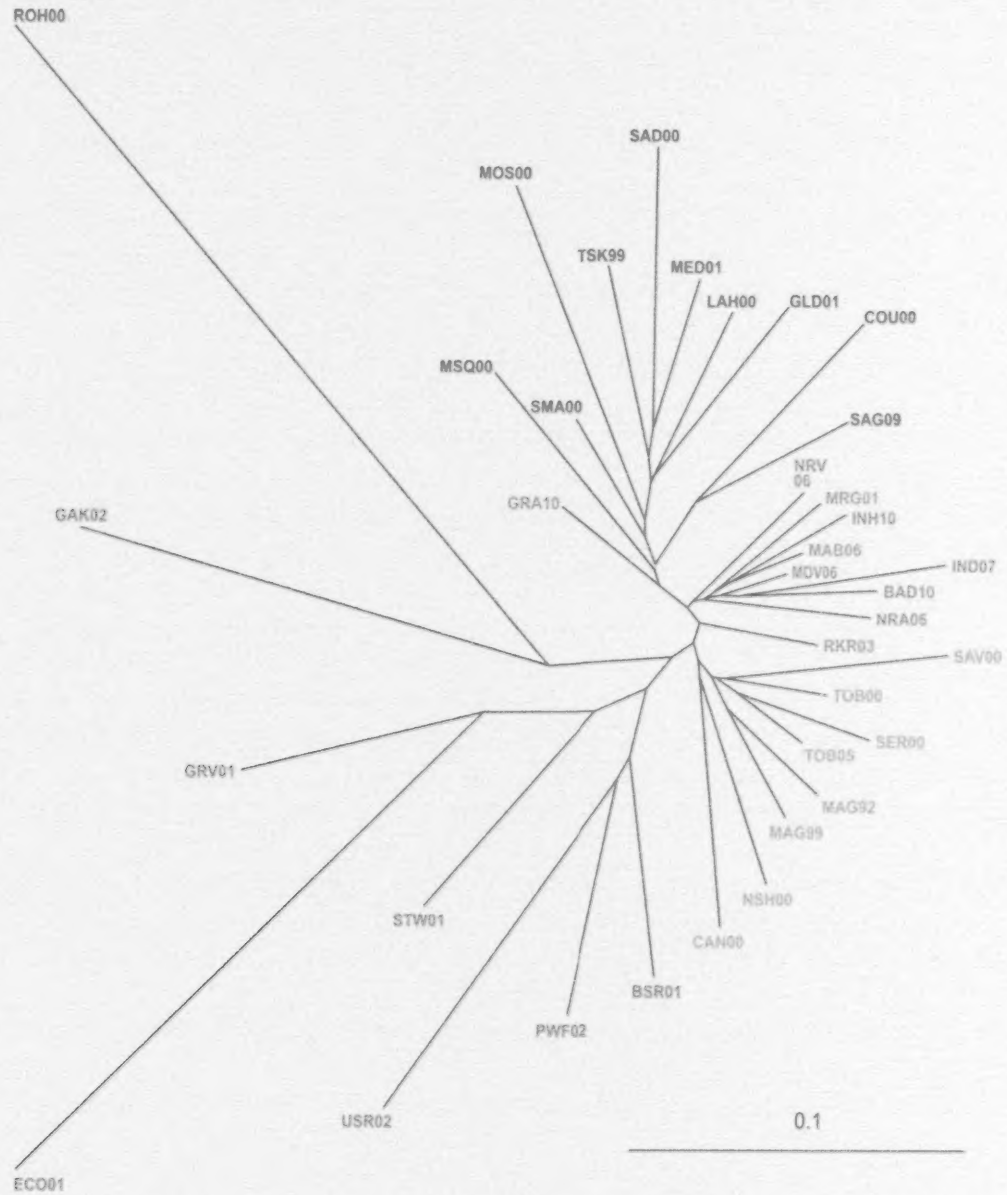

Figure 4. Phylogeny of sample collections from OBoF and other reference DUs based on Nei's D_A pairwise genetic distances estimated from 7 microsatellite loci, constructed using the neighbour-joining method. Full sample names corresponding to alphanumeric sample codes are given in Table 1.

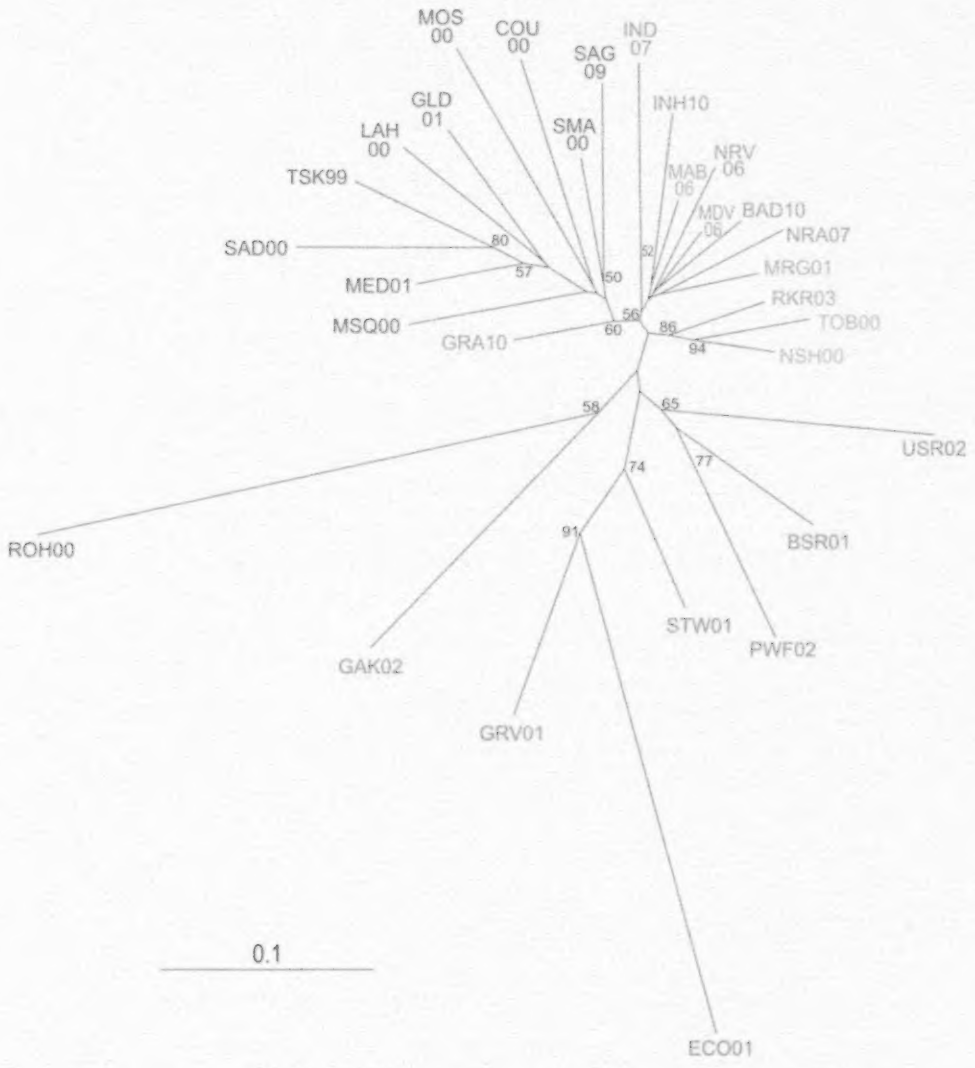

Figure 5. Phylogeny of sample collections from OBoF and other reference DUs based on Nei's D_A pairwise genetic distances estimated from 17 microsatellite loci, constructed using the neighbour-joining method. Numbers near branch nodes indicate level of support obtained by resampling across loci (with replacement) 1,000 times. Full sample names corresponding to alphanumeric sample codes are given in Table 1.

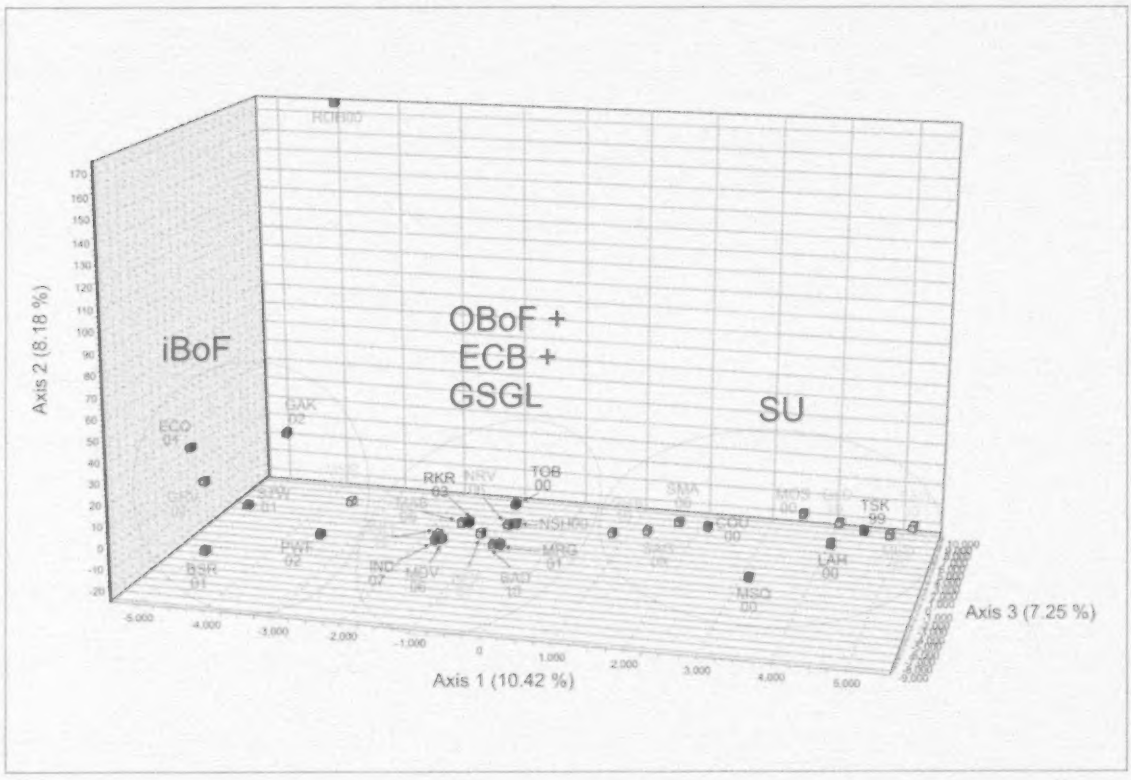

Figure 6. Factorial correspondence analysis of genotype information obtained from 17 microsatellite loci for sample collections acquired from rivers of the Outer Bay of Fundy (OBoF) and several other reference DUs, including Inner Bay of Fundy (IBoF), Eastern Cape Breton (ECB) and Gaspé-Southern Gulf of St. Lawrence (GSGL). Full sample names corresponding to alphanumeric sample codes are given in Table 1.

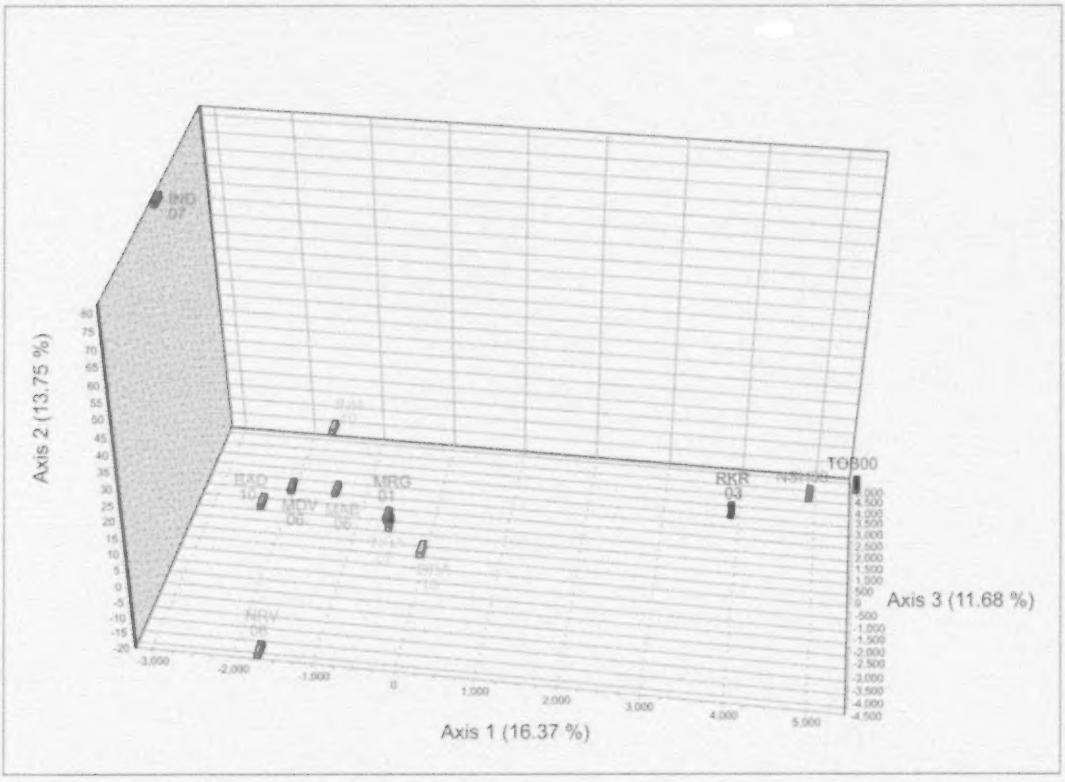

Figure 7. Factorial correspondence analysis of genotype information obtained from 17 microsatellite loci for sample collections acquired from rivers of the Outer Bay of Fundy (TOB00, NSH00), Eastern Cape Breton (GRA10, NRA07, NRV06, BAD10, MDV06, INH10 and IND07) and Gaspé-Southern Gulf of St. Lawrence (RKR03, MAB06 and MRG01) DUs. Full sample names corresponding to alphanumeric sample codes are given in Table 1.